

Standard Operating Procedure for Biochemical Oxygen Demand

1.0 Location

Biochemical Oxygen Demand determinations are performed in the Spectroscopy Lab, Room 305.

2.0 Purpose

This test is used to determine the relative oxygen requirements in wastewaters, effluents and polluted waters.

3.0 Scope

The Biochemical Oxygen Demand determination is an empirical test in which standardized lab procedures are used to determine the relative oxygen requirements of wastewaters, effluents and polluted waters. The test measures the oxygen required for the biochemical degradation of organic material and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron. It may also measure the oxygen used to oxidize reduced forms of nitrogen unless their oxidation is prevented by an inhibitor. This test is used to determine if the sample meets permit requirements before being released into the environment.

4.0 Reference

Standard Methods for the Examination of Water and Wastewater, 18th Edition, p.5-1 to 5-6, Method 5210 B. (1992).

5.0 Sample Handling and Preservation

Samples for BOD analysis may degrade significantly during storage between collection and analysis, resulting in low BOD values. Minimize reduction of BOD by analyzing the sample promptly or by cooling it to near-freezing temperature during storage. However, even at low temperatures, keep holding time to a minimum. The maximum holding time is 48 hours from time of collection. Warm chilled samples to 20 °C before analysis.

6.0 Apparatus and Materials

6.1 Incubation bottles: 300 ml \pm 3 ml capacity, with ground-glass stoppers. Clean the bottles with 10% HCl, rinse thoroughly and drain before use. As a precaution against drawing air into the dilution bottle during incubation, use a water-seal. Obtain satisfactory water seals by having excess water in the flared mouth of the BOD bottles after stopper insertion. Place a plastic cap over the flared mouth of the bottle to reduce evaporation of the water seal during incubation.

6.2 Air Incubator: thermostatically controlled at 20° \pm 2°C. Exclude all light to prevent possibility of photosynthetic production of DO.

- 6.3 Phosphate buffer solution: Dissolve 2.13 g of KH_2PO_4 , 5.44 g K_2HPO_4 , 8.35 g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ and 0.43 g of NH_4Cl in deionized water and dilute to 250 ml. Prepare monthly. Refrigerate when not in use.
- 6.4 Magnesium sulfate solution: Dissolve 22.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in deionized water and dilute to 1 liter.
- 6.5 Calcium chloride solution: Dissolve 27.5 g of CaCl_2 in deionized water and dilute to 1 liter.
- 6.6 Ferric chloride solution: Dissolve 0.25 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in deionized water and dilute to 1 liter. Solution will be light in color.
- 6.7 Acid and alkali solutions: 1N, for neutralization of caustic or acidic waste samples.
- 6.7.1 Acid: Slowly and while stirring, add 28 ml conc. sulfuric acid to DI water. Dilute to 1 L.
- 6.7.2 Alkali: Dissolve 40 g sodium hydroxide in DI water. Dilute to 1 L.
- 6.8 Sodium sulfite solution: Dissolve 0.1575 g of Na_2SO_3 in 100 ml deionized water. Prepare daily.
- 6.9 1 : 1 acetic acid - equal amounts of concentrated acetic acid and deionized water.
- 6.10 Potassium iodide solution 10%: 10 g KI/100 ml. Store in a dark bottle and refrigerate. (25 g into 250 ml water)
- 6.11 Starch solution: Prepare an emulsion of 10 g of soluble starch in a beaker or weigh boat with a small quantity of cold deionized water. Pour this emulsion into 1 liter of boiling water in a beaker and allow to boil for a few minutes. Let settle overnight. Remove scum from top and use the clear solution. Refrigerate when not in use.
- 6.12 Glucose-glutamic acid solution: Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 hour. Store in a dessicator. Add 0.1500 g glucose and 0.1500 g glutamic acid to a 1 liter volumetric flask and dilute to volume with deionized water. Prepare weekly. Refrigerate when not in use.

7.0 Procedures

7.1 Sample preparation

7.1.1 Receive samples from log-in person.

7.1.2 Determine the pH of each sample, see method I-1-11. If the pH of the sample is less than 6.8 or greater than 10, neutralize samples to approximately pH 7.0 with a solution of sulfuric acid or sodium hydroxide, see section 6.7 above. Use seeded dilution water to prepare dilutions for these samples.

7.1.3 Test for residual chlorine.

7.1.3.1 Add 5 ml of 10% potassium iodide solution and 5 ml of 1:1 acetic acid to 50 ml of sample in an erlenmeyer flask. Mix well.

7.1.3.2 Wait 10 minutes and add 1 ml of starch solution.

7.1.3.3 If the solution does not change color, there is no residual chlorine present. Go to section 7.1.5.

7.1.3.4 If the solution turns blue, there is residual chlorine present. Go to section 7.1.3.5.

7.1.3.5 Add sodium sulfite solution dropwise with mixing until the sample is clear. Record the amount of sodium sulfite needed to neutralize the residual chlorine in 50 ml of sample on BOD worksheet.

7.1.3.6 If residual chlorine is present, the sample must be dechlorinated before making dilutions. Dechlorinate the sample by adding a proportional volume of sodium sulfite (see section 7.1.3.5) to the sample volume (usually 400 ml) and use seeded dilution water to prepare the dilutions.

7.1.4 Bring samples to $20 \pm 3^{\circ}\text{C}$ and mix well before making dilutions.

7.2 Preparation of dilution water

7.2.1 Aerate reagent grade water with a resistivity of >10 megaohm - cm for approximately 4 hours.

7.2.2 Preparation of regular dilution water: immediately before use, add 1 ml each of phosphate buffer, MgSO_4 , CaCl_2 , and FeCl_3 solutions for each liter of water. Mix well.

7.2.3 Preparation of seeded dilution water: Domestic wastewater, unchlorinated or otherwise undisinfected effluents from biological waste treatment plants and surface waters receiving wastewater discharges contain satisfactory microbial populations necessary to oxidize the biodegradable organic matter in the sample. Chlorinated or otherwise - disinfected waste, high temperature wastes, or wastes with extreme pH values do not contain a sufficient microbial population. Seed the dilutions of such waste with the following treatment:

7.2.3.1 The preferred seed is effluent from a biological treatment system processing the waste. The supernatant from domestic wastewater may be used after settling at room temperature for at least 1 hour. Collected fresh for each day (on Wed, Thurs and Fri) and refrigerated until used. Determine the existence of a satisfactory population by testing the performance of the seed by doing a BOD test on the sample.

7.2.3.2 To seed the dilution water add the appropriate amount of seed per liter, example: 5 ml of seed material to 1 L of prepared regular dilution water, see section 7.2.2, and use this to fill the BOD bottles.

7.3 Analysis

7.3.1 Determine dilutions to be made: Use dilutions that result in a residual DO of at least 1 mg/l and a DO uptake of at least 2 mg/l after five days incubation. Make several dilutions of prepared sample directly in the BOD bottles, in duplicate, to obtain a DO uptake in this range.

7.3.1.1 For most routine lagoon samples three dilutions are made, 1:30, 1:10, and 1:3

7.3.1.2 For lagoon samples with a strong odor or odor of sewage, add a 1:100 dilution.

7.3.1.3 For industrial samples use a dilution range of 1:5, 1:20, 1:100, 1:500, 1:2000, or try to determine the approximate BOD from previous

samples or information received with sample and use dilutions in that range.

7.3.1.4 For dilutions greater than 1:100 make a primary dilution in a graduated cylinder before making a final dilution in the bottle.

- 7.3.2 The sample is well mixed and poured into an erlenmeyer flask (usually about 400 ml). The sample is agitated before each measurement. A 100 ml graduated cylinder is used for measuring amounts greater than 30 ml. A 10 ml graduated cylinder is used for measuring amounts of 10 ml or less. The sample is poured directly into the BOD bottles.
- 7.3.3 After the appropriate amount of sample is added to each BOD bottle, fill the bottle with regular dilution water unless seeded dilution water is needed as determined in sections 7.1.2, 7.1.3, and 7.2.3.
- 7.3.4 Measure the initial DO on each bottle using the membrane electrode method, see section I-1-9 of the methods manual. The DO of the sample should read between 7.5 and 9.0 mg/L. Aerate or shake to obtain this level. Record on BOD worksheet.
- 7.3.5 Stopper tightly with no bubbles, waterseal, and incubate for 5 days at 20°C in the dark.
- 7.3.6 Prepare a regular dilution water blank and a seeded dilution water blank and incubate with samples for 5 days at 20°C.
- 7.3.7 Prepare dilutions of 1:100, 1:50, 1:20 of the raw sewage used as seed material to determine its BOD. This value is needed to calculate the seed correction factor used in calculations for all seeded samples.
- 7.3.8 Glucose-Glutamic Acid check: Because the BOD test is a bioassay, the results can be influenced greatly by the presence of toxicants or by use of a poor quality seeding material. For these reasons a glucose-glutamic acid check is performed daily.
- 7.3.8.1 Prepare two 1:50 dilutions of the glucose-glutamic acid solution with seeded dilution water.

7.3.8.2 The results should read 200 ± 30 mg/L BOD. If results fall outside of this range, determine problem and take measures to correct. Determine if seeded samples are valid. Notify your supervisor before reporting values.

7.3.9 After 5 days determine the DO in sample dilutions, blanks, and checks using the membrane electrode method and record on BOD worksheet.

7.3.10 See section 9.0 for calculations.

8.0 Quality Control

8.1 Perform glucose-glutamic acid check. See section 7.3.8.

8.2 All samples are prepared in duplicate.

9.0 Data Analysis

9.1 When dilution water is not seeded:

$$\text{BOD, mg/l} = (D1 - D2) * F$$

9.2 When dilution water is seeded:

$$\text{BOD, mg/L} = [(D1 - D2) - S] * F$$

Where:

D1 = DO of diluted sample immediately after preparation, mg/l.

D2 = DO of diluted sample after 5 days incubation, mg/l.

F = Dilution factor of sample used. For 1:4 dilution, F=4.

S = Seed correction factor =

BOD of raw sewage x amount of seed/1000ml x fraction of dilution water used.

Example:

BOD of seed = 180, dilution 1:50, 5 ml seed to 1000 ml dilution water

$$S = \frac{180 \times 5 \text{ ml} \times 49}{1000 \times 50}$$

- 9.3 If more than one sample dilution meets the criteria of residual DO of at least 1 mg/l and a DO depletion of at least 2 mg/l and the dilution water blank does not deplete by more than 0.5 mg/l, average all results in the acceptable range. If the dilution water blank depletes by more than 0.5 mg/l use the results of the dilution with the highest sample concentration.
- 10.0 Documentation
 - 10.1 pH values are recorded on worksheet in BOD pH logbook.
 - 10.2 Residual chlorine information is recorded on the BOD worksheet.
 - 10.3 The initial and 5 day DO, seed correction, and other calculations are recorded on the BOD worksheet found in the BOD logbook.
 - 10.4 Store printouts of results from LMS in appropriate books in BOD area.
- 11.0 Records

All worksheets and print-outs are stored in proper books in BOD area of room 305.
- 12.0 Wastewater Biological Hazards
 - 12.1 Introduction
 - 12.1.1 Wastewater or sewage has a wide variety of potential disease causing agents called pathogens. These agents include bacteria, fungi, protozoa and viruses.
 - 12.1.2 Good personal hygiene practices are important in the control and spread of disease from contact with wastewater. Most infections are caused from hand to mouth contact. Avoid eating, touching your eyes or body with contaminated hands. Hands should be washed often with a disinfectant soap and plenty of hot water. Get proper medical attention to avoid infection from cuts or puncture wounds when in contact with wastewater. Always cover cuts with a band-aid and wear gloves.
 - 12.1.3 Tetanus vaccinations are highly recommended for all workers who will be exposed to wastewater.
 - 12.2 Personal Protective Equipment

- 12.2.1 Clothing creates a barrier between you and the disease causing agent. Wear your lab coat at all times when working in this area.
- 12.2.2 Latex gloves are recommended when working with wastewater.
- 12.2.3 Safety glasses or shields must be worn at all times.
- 12.3 Disinfection of work surfaces
 - 12.3.1 Clean work surfaces with recommended antibacterial agent -Listophene- (4ml of concentrate/500ml water) at least once a day or more often as needed.
 - 12.3.2 Spray sinks, handles, work surfaces.
 - 12.3.3 Empty liquid waste containers immediately after use, rinse and spray with listophene solution.